GLOMALIN: A SOIL PROTEIN IMPORTANT IN CARBON SEQUESTRATION

Sara F. Wright[1], Matthias C. Rillig[2] and Kristine A. Nichols[3]

[1]USDA-ARS-SMSL, Building 001, Room 140, BARC-W, Beltsville, MD 20705, [2]University of Montana, Division of Biological Sciences, HS104, Missoula, MT 59812, [3]University of Maryland, Department of Natural Resources and Landscape Architecture, College Park, MD 20742

KEYWORDS: Aggregate stability, soil organic matter, soil stability, glycoprotein

ABSTRACT

Elevated atmospheric CO_2 levels lead to greater fixation of carbon by plants and greater transfer of carbon to roots and soil. We are studying the amplification of a series of events that flow from increased inputs of carbon to plant roots and subsequently to sequestration of organic carbon in soil aggregates. Soil aggregates are groups of primary particles that adhere to each other more strongly than to surrounding soil particles. Plant roots provide carbon for growth and reproduction of a ubiquitous group of symbiotic fungi called arbuscular mycorrhizal fungi (AMF). A recent discovery shows that AMF produce copious amounts of an insoluble, hydrophobic, recalcitrant glycoprotein, named glomalin, which is important in stabilizing soil aggregates. Aggregates store and protect additional organic carbon until the aggregates break down. Thus, greater stability of aggregates leads to larger amounts of protected organic carbon in terrestrial ecosystems.

INTRODUCTION

Increases in atmospheric CO_2 (Keeling et al., 1995) highlight the need to explore ways to trap and sequester this greenhouse gas in terrestrial ecosystems. Plants fix CO_2 and allocate a part of the photosynthate to roots (Rogers et al., 1994) and soil (Jones et al., 1998). Organic carbon in soil plays an important role in soil aggregation (Kemper & Rosenau 1986). Soil aggregates are groups of primary particles that adhere to each other more strongly than to surrounding soil particles (Martin et al., 1955). Relatively labile carbon is protected in soil aggregates (Cambardella & Elliott 1992; Jastrow & Miller 1997; Six et al., 1998) and has a turnover of 140 – 412 years in a pasture soil, depending upon the aggregate size (Jastrow et al., 1996).

Increased fixation of CO_2 by plants may have a direct effect on root symbionts that utilize plant-fixed carbon for growth – the arbuscular mycorrhizal fungi (AMF). AMF are ubiquitous symbionts of the majority of land plants. AMF are also important in soil aggregate stabilization (Tisdall & Oades, 1982; Jastrow & Miller, 1997). The contribution of AMF to stabilization of aggregates was thought to be through entrapment of soil particles by fungal hyphae, the filamentous structures making up the body of the fungus. Hyphae extend several centimeters from the root into soil. Estimates of AMF extraradical hyphae vary widely (Rillig & Allen, 1999), but one of the highest estimates is 111 m cm^3 in a prairie grassland soil (Miller et al., 1995).

A recent discovery of copious production of a glycoprotein, glomalin, by hyphae of AMF (Wright et al., 1996) and its role in aggregate stability (Wright & Upadhyaya 1998) has implications for enhanced carbon sequestration by soils under elevated CO_2. Recently Rillig et al. (1999) provided evidence for a change in aggregate stability under elevated CO_2 in three natural ecosystems. Increases in hyphal length and glomalin concentration in soils were shown concurrently.

This report will review glomalin and aggregate stability: (i) in disturbed and undisturbed agricultural soils, (ii) in agricultural soils in transition from plow- to no-tillage, (iii) in natural grasslands under increased CO_2 and (iv) in a tropical soil. We will also present characteristics of glomalin that indicate the unusual nature of the molecule.

MATERIALS AND METHODS

Glomalin extraction. Extractions were performed as described by Wright & Upadhyaya (1998). One-gram samples of air-dried soil were placed in 8 mL 20 mM citrate, pH 7.0 and autoclaved (121 °C) for 30 min to remove the easily-extractable glomalin (EEG). After centrifugation (10,000 x g) and removal of the supernatant, 8 mL 50 mM citrate, pH 8.0 was added to the remaining soil and heated at 121 °C for 60 min to extract total glomalin (TG). Additional extractions with 50 mM citrate were done until the supernatant was a straw color, indicating that glomalin, a red-brown color, had been removed. One mL of EEG was removed and then the remaining supernatant containing EEG was combined with all of the supernatants from the 50 mM citrate extractions. Protein was determined by the Bradford dye-binding assay with bovine serum albumin as the standard (Wright et al., 1996). An indirect enzyme-linked immunosorbent

assay (ELISA) was used to quantify the immunoreactive fraction (IREEG and IRTG). Weight of soil was corrected for non-aggregated coarse material.

Glomalin purification. Glomalin was precipitated with trichloroacetic acid (TCA) and then dialyzed against 10 mM borate, pH 8.0 as described by Wright et al. (1998). Dialyzed samples were freeze-dried.

Aggregate stability. The apparatus described by Kemper & Rosenau (1996) was used todetermine water stability of air-dried aggregates. Air-dried bulk soil was sieved to remove the 1 – 2 mm and 0.5 – 1 mm aggregates. Four g of aggregates was placed in a sieve and pre-wetted by capillary action. The 1 – 2mm aggregates were in 0.25 mm sieves, and the 0.5 – 1 mm aggregates were in a 0.01 mm sieve. Aggregates were pre-wetted by capillary action and then tumbled for 5 min in a column of water. After drying remaining aggregates at 70 °C aggregate stability was calculated: % stability = (g aggregates remaining on the sieve - the coarse material/ 4 g - coarse material) x 100.

Characterization of glomalin. Various routine and specialized assays have been performed to characterize the glomalin molecule. Routine assays are Bradford protein and enzyme-linked immunosorbent assay (ELISA). Methods for these are described above. Sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel electrophoresis banding patterns on 12% T gels stained with silver are also run on a routine basis (Wright and Upadhyaya, 1996). Iron was analyzed by atomic absorption spectroscopy after microwave digestion in nitric acid.

RESULTS

We have studied undisturbed, disturbed, and soils under elevated CO_2 for the relationship between aggregate stability and glomalin (Rillig et al., 1999; Wright & Upadhyaya, 1998; Wright et al., 1999) in temperate regions. In general, undisturbed soils have the highest aggregate stability and glomalin, but soils appear to differ in the amount of glomalin they can accumulate (Table 1). Both aggregate stability and glomalin are higher in undisturbed compared with disturbed soils (Tables 2 and Fig. 1).

TABLE 1: Selected undisturbed soils that illustrate the range in values for aggregate stability and measures of easily extractable glomalin (EEG), total glomalin (TG), and the immunoreactive fractions of each (IREEG and IRTG, respectively).

Location	Soil Type (series)	Aggregate Stability (%)	TG (mg/g)	IRTG (mg/g)	EEG (mg/g)	IREEG (mg/g)
Maryland	Silt loam (Baltimore)	80	5.2	4.4	2.9	2.5
Virginia	Silt loam (Georgeville)	93	14.1	10.9	10.0	8.3
Illinois	Silty clay loam (Sable)	52	12.6	5.7	4.3	1.9
Minnesota	Sand (Nymore)	55	4.7	8.6	5.8	4.5
Texas[1]	Sandy loam (Berta, Posey)	22	3.3	1.5	1.5	0.6
Colorado[1]	Silt loam (Weld)	60	3.0	1.7	1.0	1.0

[1]Alkaline soils. All other soils are acidic.

Cultivated soils have been compared with undisturbed soil under native or introduced grasses to determine the effects of disturbance on glomalin. The immunoreactive easily extractable (IREEG) fraction of glomalin is most closely correlated with aggregate stability across soil types and locations. This fraction is similar to glomalin on fresh hyphae using currently available analytical procedures (Wright & Upadhyaya, 1998) (Table 2).

TABLE 2: Immunoreactive easily extractable glomalin (IREEG) and aggregate stability of 1 –2 mm aggregates for three geographic locations with comparisons between disturbed and undisturbed sites (SD in parentheses).

Location	Soil Type	Disturbed		Undisturbed	
		Aggregate stability (%)	IREEG[1] (mg/g)	Aggregate stability (%)	IREEG (mg/g)
Texas[1]	Sandy loam	8.6 (0.6)	0.4 (0.2)	22.3 (3.5)	0.6 (0.2)
Colorado[1]	Silt loam	11.1 (4.3)	1.1 (0.3)	59.9 (20.1)	1.7 (1.4)
Maryland[1]	Silt loam	18.1 (3.0)	0.6 (0.0)	58.9 (3.5)	1.9 (0.3)

[1]Texas – three cultivated sites were compared with three nearby rangeland sites; Colorado – 45 cultivated plots were compared with nine nearby individual grass soils; Maryland – four cultivated plots were compared with four sites from a grass buffer surrounding the plots.

Soil management affects aggregate stability and glomalin. A recent study compared no-tillage to plow-tillage over three years for a silt loam soil in Beltsville, MD corn plots (Wright et al, 1999). Both aggregate stability and glomalin increased during the transition (Fig. 1).

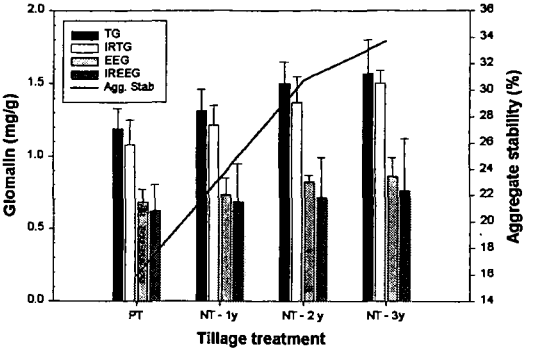

FIGURE 1: Changes in aggregate stability and glomalin during transition from plow- to no-tillage for corn production. TG = total glomalin, EEG= easily extractable glomalin and IR= immunoreactive fraction.

A chronosequence (300 years – 4.1 million years) from Hawaii was studied (manuscript in preparation) to determine glomalin levels in a tropical climate. Very large amounts of TG were found (Fig. 2).

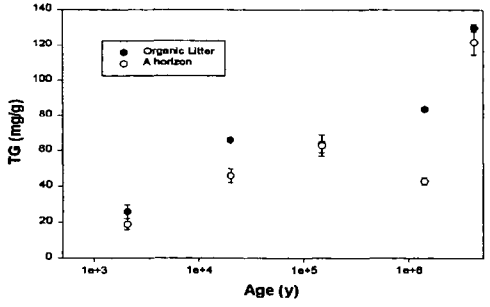

FIGURE 2: Total glomalin in the upper layers of an organic soil chronosequence from Hawaii.

Exposure of annual grassland to elevated CO_2 resulted in increases the number of aggregates as a proportion of soil mass, and increases in aggregate stability and glomalin (Rillig et al., 1999). Results of this study are summarized in Table 3.

TABLE 3: Effects of increased CO_2 on aggregates as a percent of bulk soil, water stability of aggregates, and glomalin in natural grasslands in California (Rillig et al., 1999).

	Sandstone		Serpentine	
	Ambient CO_2	Increased CO_2	Ambient CO_2	Increased CO_2
Aggregates 1-2 mm (% of soil)	14.4 (0.5)	15.1 (0.3)	16.7 (0.6)	18.1 (0.5)
Aggregates 0.25-1mm (% of soil)	17.1 (0.6)	20.0 (0.8)	26.9 (1.6)	25.6 (1.1)
Water stable 1-2 mm (%)	86.7 (1.6)	90.1 (1.3)	76.2 (1.5)	81.0 (1.3)
Water stable 0.25-1 mm (%)	88.9 (0.9)	92.8 (0.6)	84.3 (0.9)	85.3 (0.5)
Immunoreactive glomalin (mg/g)	0.7 (0.0)	0.8 (0.0)	1.0 (0.0)	1.1 (0.0)

Glomalin is categorized as a glycoprotein (Wright et al., 1998). Carbon, nitrogen, and iron in glomalin from hyphae of two AMF and a soil and other soil are shown in Table 4.

TABLE 4: Total carbon, iron and nitrogen in selected glomalin samples.

Assay	Baltimore soil	Glomus intraradices UT126	Gigaspora rosea FL225
Total Carbon (%)	26.4	24.80	17.50
Fe (%)	7.2	0.5	0.5
N (%)	1.63	1.70	1.22

Similarity between glomalin on hyphae from axenic cultures and soil indicates that the substance extracted from soil is the same as that on hyphae. Raw extracts from hyphae or soil show the same banding patterns on SDS-PAGE (Fig. 3).

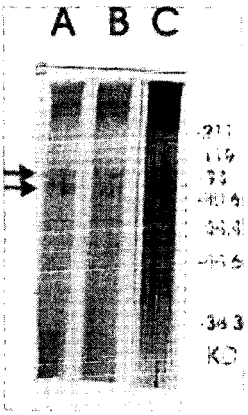

FIGURE 3: SDS-PAGE of glomalin extracted from hyphae of *Glomus intraradices* UT126 (A), *Gigaspora gigantea* MA453A (B), and Baltimore soil (C). Molecular weight standards (KD) are indicated. The arrow indicates the position of two bands.

DISCUSSION

Glomalin, a major component of previously unidentified organic matter in soil, reaches levels of 1.4 – 13% of air-dried mineral soils based on Bradford protein values (Table 1, Fig. 2). Comparison of amounts of humic and fulvic acids and glomalin from Hawaiian organic soil on a weight basis show that glomalin can be as high as 26% of the soil while fulvic, humic, and the mineral fraction are 2.7, 6.7, and 60.7%, respectively (unpublished data).

Levels of glomalin in soil are closely associated with aggregate stability. This was shown in undisturbed soils, undisturbed compared with disturbed soils, and during transition from plow- to no-tillage (Tables 1 and 2, Fig. 1). Since glomalin production appears to be directly linked to carbon supplied by plants, production of glomalin may be affected by increased atmospheric CO_2 (Table 3). Evidence for this was also found in a sorghum field equipped with a free-air CO_2 enrichment (FACE) system (manuscript in preparation). Measures of soil AMF hyphal length showed a strong response to CO_2 as well as one fraction of glomalin. Glomalin and AMF hyphal lengths were positively correlated with soil aggregate stability.

The structure of glomalin at present unknown, but the molecule is insoluble, hydrophobic and recalcitrant. Insolubility of the molecule is probably the reason that glomalin was not discovered until recently. We have seen evidence of insolubility and sloughing of glomalin from AMF hyphae in sand cultures of these fungi on plant roots. When cultures are harvested and fresh roots are placed in water, glomalin floats to the surface of the water and collects as a film at the air-water interface. This film entraps air bubbles and adheres to plastic surfaces. Plastic traps have been used to quantify glomalin in cultures (Wright & Upadhyaya, 1999). Residence time of glomalin in soils is currently being investigated, but preliminary results indicate that glomalin in the undisturbed Hawaiian soils (Fig. 2) lasts for 6 – 42 years (manuscript in preparation).

Other fungal proteins, hydrophobins, have characteristics similar to glomalin (Wessels, 1996). For example, the hydrophobin SC3 produced by the basidomycete *Schizophyllum commune* is a glycoprotein (Wessels, 1997) that is insoluble in hot SDS and forms insoluble complexes (de Vries et al., 1993). Interfacial self-assembly of SC3 monomers leads to insoluble amphipathic films about 10 mm thick that coat air bubbles (Wessels, 1996). SC3 is secreted from hyphal tips as a monomer and then flows over the hyphal surface as semi-fluid polymers (Wessels, 1996). Hydrophobic interactions of monomers and other bonds may contribute to the overall hydrophobicity of the molecules (Wessels, et al., 1991). Purified SC3 is described as a milky suspension (Wessels, 1997). However, there are no reports of any iron-containing hydrophobins at this time.

Iron in or on glomalin (Table 4) imparts a red-brown color to extracted glomalin, and we think that iron is critical to the stability of the molecule in soil and in laboratory proccdures used to reveal the molecular structure. Hydrolysis for amino acid analysis requires 24 h at 150 °C, and a pre-treatment to remove some of the iron may be necessary to achieve complete hydrolysis. We

have successfully removed a large amount of iron from glomalin using 8-hydroxy quinoline, but weaker chelators are slightly effective to ineffective for removal of iron. Lactoferrin is an iron-bearing protein with similarities to glomalin. Lactoferrin is a member of a group of iron-binding glycoprotein called transferrins. Althought this glycoprotein is found in animals, it shares some intriguing characteristics with glomalin. Both are approximately the same size (~80 – 90 KD), extracellular, heat and enzymatically stable and difficult to hydrolyze. Lactoferrin hyperaccumulates iron and undergoes conformational changes upon binding iron. We have evidence that glomalin hyperaccumulates iron (Table 4) and are working on dissecting the conformational changes that may occur. Lactoferrin functions in iron binding and transport and as a bacteriostatic molecule – roles that glomalin may play in the soil environment.

Currently we are working to determine amino acid and carbohydrate contents and carbohydrates in glomalin from axenic cultures and soils. This requires stripping the iron with 8-hydroxy quinoline (manuscript in preparation).

CONCLUSIONS

Glomalin is abundant in soils and is closely correlated with aggregate water-stability. Glomalin contains carbon and hence constitutes a non-trivial portion of the terrestrial carbon pool. Possibly far more importantly, however, stabilization of aggregates amplifies the role of glomalin in soils because carbonaceous compounds are protected from degradation inside of aggregates. Increased atmospheric CO_2 can lead to increased production of glomalin because of the symbiotic association that exists between plants and producers of glomalin, AMF. We have also shown that glomalin concentrations in soils are influenced by management practices, for example in agroecosystems, further highlighting the role of this protein in carbon storage. Glomalin is an unusual molecule that has proven difficult to analyze biochemically due to its recalcitrance and possible complexity. Future research will be directed towards the elucidation of its structure and the controls on its production.

REFERENCES

Cambardella, C. A., & Elliott, E. T. (1992) *Soil Sci. Soc. Am. J. 56*, 777-783.

De Vries. O. M. H., Fekkes, M. P., Wösten, H. A. B., & Wessels, J. G. H. (1993) *Arch. Microbiol. 159*, 330-335.

Jastrow, J. D., & Miller, R. M. (1997) Soil Processes and the Carbon Cycle, pp 207-223, CRC Press, Boca Raton, FL.

Jastrow, J. D., Boutton, T. W., & Miller, R. M. (1996) *Soil Sci. Soc. Am. J. 60*, 801-807.

Jones, T. H., Thompson, L. J., Lawton, H. H., Bezemer, T. M., Bardgett, R. D., Blackburn, T. M., Bruce, K. D., Cannon, P. F., Hall, G. S., Hartley, S. E., Howson, G., Jones, C. G., Kampichler, C., Kandeler, E., & Ritchie, D. A. (1998) *Science 280*, 441-443.

Keeling, C. D., Whorf, T. Pl, Whalen, M., & van der Plicht, J. (1995) *Nature 375*, 666-670.

Kemper, W. D., & Rosenau, R. C. (1986) Methods of Soil Analysis (Part I), pp. 425-442, American Society of Agronomy, Madison, WI.

Martin, J. P., Martin, W. P., Page, J. B., Ranley, W. A., & De Ment, J. D. (1955) *Adv. Agro. 7*, 1-37.

Miller, R. M., Reinhardt, D. R., & Jastrow, D. D. (1995) *Oecologia 103*, 17-23.

Rillig, M. C., & Allen, M. F. (1999) *Mycorrhiza 9*, 1-8.

Rillig, M. C., Wright, S. F., Allen, M. F., & Field, C. B. (1999) *Nature 400*, 628.

Rogers, H. H., Runion, G. B., & Krupa, S. V. (1994) *Environ. Pollution 83*, 155-189.

Wessels, J. G. H. (1996) *Trends Plant Sci. 1*, 9-15.

Wessels, J. G. H. (1997) *Adv. Microbial Physiol.* 38,1-45.

Wessels, J. G. H. de Vries, O. M. H., Ásgeirsdttir, & Schuren, F H. J. (1991) *Plant Cell 3*, 793-799.

Six, J., Elliott, E. T., Paustian, K., & Doran, J. W. (1998) *Soil Sci. Soc. Am. J. 62*, 1367-1377.

Tisdall, J. M., & Oades, J. M. (1982) *J. Soil Sci. 33*, 141-163.

Wright, S. F., & Upadhyaya, A. (1996) *Soil Sci. 161*, 575-586.

Wright, S. F., & Upadhyaya, A. (1998) *Plant Soil 198*, 97-107.

Wright, S. F., & Upadhyaya, A. (1999) *Mycorrhiza 8*, 283-285.

Wright, S. F., Upadhyaya, A., & Buyer, J. S. (1998) *Soil Biol. Biochem. 30*, 1853-1857.

Wright, S. F., Starr, J. S., & Paltineanu, I. C. (1999) *Soil Sci. Soc. Am. J. 63*, 1825-1829